GROWTH OF TIMOTHY (*PHLEUM PRATENSE* L.) SEED FROM POLLINATION TO MATURITY AND EFFECT OF DEGREE OF MATURITY AT HARVESTING AND OF CHILLING UPON GERMINATION AND SEEDLING VIGOUR

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The undersigned hereby certify that they have read and recommend to the School of Graduate Studies for acceptance, a thesis entitled, "Growth of Timothy (*Phleum pratense* L.) Seed from Pollination to Maturity and Effect of Degree of Maturity at Harvesting and of Chilling upon Germination and Seedling Vigour", submitted by Phillip Edward Meric Leith, D.F.C., B.S.A., B.Com., in partial fulfilment of the requirements for the degree of Master of Science.

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I N T R O D U C T I O N

A crop grown for seed must be harvested at the right stage of growth if the greatest possible seed yield is to be obtained. Thus, as high a percentage as possible of the seeds must have reached such a stage of growth at the time of cutting that they will later produce seedlings of high vitality. The danger of cutting too late must also be guarded against. While cutting too early will result in underdeveloped seed, seed left on the flower head for too long a period will result in shattering and a considerable loss in the seed yield per acre. Thus a record of the course of seed growth and the effect upon seed quality of premature harvesting should aid in determining the time at which a crop must be cut in order to obtain the maximum yield of seed and the optimum quality. A considerable amount of work has been carried out on the development of cereal seed but enquires into the growth of forage plant seeds have not been extensive.

It was therefore decided to carry out experiments on the growth of timothy seed since it is a grass that has a very wide distribution and use.

The aims of the investigation were as follows:

1. To study the growth of the seeds from pollination to maturity so that stages of seed growth could be defined. It was decided that the growth of the seed could be followed by observing changes in fresh weight, dry weight and water content.

2. To harvest seeds at different stages of growth and to observe their germinating capacity, the incidence of dormancy and the vigour of seedling growth.

3. To carry out such preliminary studies on the pollination and self-fertility as were necessary for undertaking the above projects.

REVIEW OF LITERATURE

Hyde (1950) obtained white clover seed of known age some of which he used to determine the average weight and water content while the remainder was dried and kept for germination studies. He recognized three stages in the development of the seed, which from pollination to ripeness occupied about twenty-six days. These three stages were listed as growth, food reserve accumulation and ripening.

Hyde studied the effect on seed quality through harvesting at different stages of seed development, and lists viability, seedling vigour and storage life as the aspects of most interest. The viability, obtained by him, was six and ninety percent respectively in seeds harvested ten and fifteen days after flowering.

He stated that vigour is represented in speed of germination, size of seedling, rate of seedling growth, and depth of the soil through which the seedling can emerge. Samples of seed which he harvested at different stages of development were scarified and placed in the incubator, the speed of germination increasing slightly with advancing age at harvest, ninety percent of the viable seeds germinating

in three and a half days for seed harvested twelve days after flowering and in two days for mature seed.

Seed harvested twelve days after flowering gave seedlings, which after growing for six weeks in a greenhouse had approximately half the fresh weight of those from mature seed. Hyde believed that the most satisfactory indicator of maturity is seed weight and he recommended its use in New Zealand for judging seed quality.

Whyte (1946) stated that growth denotes the increase in weight and volume of the plant at any particular stage, while development consists of certain stages followed in serial order, these stages being described by Lysenko (1935) as "qualitative" changes occurring in the plant, each of which must be traversed before seeding can take place. The complexes of external conditions necessary for the plant to pass through the developmental stages and those necessary for the growth of the plant at any particular developmental stage are often not identical, not only as regards the factors required but also as to their intensities. Consequently this distinction between the conditions required for growth and development make it possible to have conditions which lead to any of the following states; (a) rapid growth of the plants but slow development (b) slow growth and rapid development (c) rapid growth and rapid development.

Lysenko (1935) distinguished between the "morphological" changes occurring during plant development and the "qualitative" stages. The two may or may not coincide, thus some qualitative changes may be associated with morphological, though morphological changes do not necessarily imply qualitative stages. The stages require for their completion, a definite set of external factors such as temperature, light, humidity and aeration. Frequently, one factor may be insufficient to meet the requirements of a particular stage of development. Thus the thermoperiod and photoperiod have both been named with reference to their limiting factors. The application of vernalization attempts to provide artificially those conditions necessary to excelerate the time required for maturity to be reached.

Lysenko (1935), experimenting mainly with cereals, found that the processes conditioning their sexual reproduction may occur not only in growing plants, but also in a seed with an embryo which has just commenced development but not broken the seed coat. This principle forms the basis of vernalization in which the thermo-stage is effected not in growing plants, but in seeds which have just begun their development.

Hyde (1950) explained what meaning he attached to the terms maturity and ripeness. He stated that seed is mature when it has acquired its maximum dry weight and that it is spoken of as being ripe when it has dried out to a moisture

content in equilibrium with the atmosphere.

Regarding the relation of the stage of seed maturity to dormancy, Thompson (1935) found that immature lettuce seed gave a marked increase in germination following exposure to light but that mature seed showed little increase. He suggested that plant nutrition affects the number of flowers and seeds produced by individual plants which in turn is correlated with dormancy. Kearns and Toole (1939) found that freshly harvested and immature seed of Festuca required a low temperature, but that as the seed aged it germinated over a wider range of temperature. Sprague (1936) made periodic samplings of immature seed corn and found that normal germination did not occur until the moisture content had been reduced to 25% or less. Timothy seed, according to Toole (1939), when freshly harvested was partially dormant, and after-ripening occurred more rapidly in cut heads than in threshed seed.

Whyte (1946 and 1948) reported that Gregory and Purvis (1938) obtained seeds of winter rye and winter wheats only five days after fertilization that were capable of germinating and ultimately producing perfectly normal plants. Nutman (1941) confirmed these observations of Gregory and Purvis (1938) with rye and similar ones made earlier by Harlan and Pope (1922 and 1926) with barley.

Whyte (1946) stated that an embryo which has not entered the dormant state may be as sensitive to vernalization as an embryo brought from the resting condition by soaking. He also reported that dormancy is characterized by a fall in the vital activity of the embryo to a minimum, and by a maximum increase in its insensitivity to environmental conditions. He reported that low temperatures are said to act only on ripening grain, which are in the milk-ripe or wax-ripe state and contain an active embryo. Capacity to react decreases as dormancy approaches.

The Reports of the Special Committee on Standardized Tests (1943-4) show that in 1944 the Committee on Standardized Tests of the Association of Official Seed Analysts adopted the following definition of seed germination: "In seed laboratory practice, germination is defined as the emergence and development from the seed embryo of those essential structures which for the kind of seed in question, are indicative of the ability to produce a normal plant under favourable conditions". This definition was also approved and adopted by the United States Department of Agriculture. Porter (1949) stated that botanically, a seed is a ripened ovule, which implies a completely developed structure capable of reproducing its kind.

Kains and McQuesten (1951) stated that germination or sprouting is the resumption of growth by the dormant

embryo or young plant in the seed, it being complete when growth has ruptured the seed coats and the embryo has emerged. According to Hill et al. (1936) a seed has germinated when the radicle has emerged from the seed coat, but that germination is usually considered complete only when the seedling has become an independent plant.

Barton and Crocker (1948) defined dormancy as a general term which may be applied to any condition which prevents germination when ordinary requirements of moisture and temperature have been met. They report that, as concerns seeds, it has been applied most often to the condition of the embryos or endosperms, or both, of many temperate-zone plants which will germinate only following a period of after-ripening in a moist medium at low temperature. Weaver and Clement (1929) stated that dormancy is usually more pronounced in seeds produced in late summer or autumn.

Porter (1949) stated that environmental conditions which facilitate germination include soaking seeds prior to germination, alternate wetting and drying, coat-disintegrating action of soil agents, drying the seeds and alternating temperatures. He notes that the requirements for germination depend on age, storage temperature and humidity, response to light and oxygen, and that tap water sometimes contains chemicals that are injurious to germination. Shuck (1935) reported that repeated growth of batches of lettuce seeds on the

same filter paper leads to the accumulation of inhibiting substances on the paper that inhibit later sowings.

Crocker (1948) believed that a period of dry storage may be quite as important a factor in after-ripening dormant seeds as is low-temperature stratification, since most seeds of cultivated grains and other grasses after-ripen in dry storage.

Kains and McQuesten (1951) defined after-ripening as the term applied to the period between the maturity of the fruit of certain plants and the time when the seeds will germinate. After-ripening is defined by Barton and Crocker (1948) as a general term which embraces the changes which take place inside the seed, making it ready to grow when planted in the ordinary manner. They report that these changes may involve actual growth and development of the embryo itself, and that they may, and usually do, include increasing acidity and altered enzyme activity within the tissues of the embryo. Crocker (1948) reported Barton (unpublished work) as having found that certain seeds that after-ripen in dry storage increase greatly in the initial rate of water absorption and somewhat in the final total amount held by them when fully imbibed as the dry storage period increases.

Hill et al. (1936) defined stratification as the placing of seeds between layers of sand, sawdust, peat, or other material and keeping them moist and at a low temperature. They report that it has been found that many seeds can be

made to germinate if stratified at low temperatures, a temperature range of 0 to 10°C having been found the most effective for the majority of species studied. Kains and McQuesten (1951) and Crocker (1948) report that the Boyce Thompson Institute has proved mixing to be far better than stratification, though this term is still used for the new method. Crocker (1948) stated that a single period of moist low-temperature stratification by imitating nature's methods of after-ripening seeds in the temperate zone causes some seeds with dormant embryos and some with non-dormant embryos to germinate.

Porter (1949) believed that the most important of the factors concerned with the longevity of seeds are stage of maturity when harvested, viability, kind of seed (including genetic factors), moisture content of the seed when stored, air temperature and humidity in the storage room, rate of water absorption or loss by the seed in storage, respiration rate of different temperatures and atmosphere in which the seed is stored. Lafferty (1931) reported that when seeds were stored in paper bags at room temperature, a small but gradual decrease in germination occurred the first few years, followed by rapid deterioration until all were dead. The number of years required for complete loss in vitality was nine for oats, meadow fescue and timothy, and fourteen for white clover. Boswell et al.(1940) found that a humidity between 45% and 50% was a safe level for most kinds of seeds.

Hollowell and Tysdal (1948) stated that the seed yields of legumes and grasses may be adversely affected by unadapted varieties, poor cultural and management practices, diseases, unfavourable weather, soils deficient in major or minor nutritive elements, harmful insects, lack of beneficial insects, and losses in harvesting. They believed that, in harvesting seed, time and method are the important factors, and that the degree of shattering, length of blooming and seed-setting period, and the amount and conditions of growth differ widely among legumes and grasses.

M A T E R I A L S

Three clonal plants of both H. 77/30 Aberystwyth (pasture-hay type) and H. 62/26 Aberystwyth (hay type) were removed from a plot at Grasslands Division, Palmerston North on the 22nd November 1950. Each of these clonal plants were then divided into four sections. By this method, twelve clonal plants of each of the two types of timothy were procured. The reason for this procedure was to assure that all seed resulting from the cross-pollination between any two plants would be as similar as could be obtained under a crossing program.

The twenty-four plants were put into eight inch pots which were then placed under a portico. The H. 77/30 Aberystwyth clonal plants, although from a pasture-hay type, were more erect in their growth form than were the plants of H. 62/26 Aberyswyth which were from a hay type. The leaves of the plants of H. 62/26 were rougher than those of the plants of H. 77/30. After transplanting, the H. 62/26 plants required three days to return to their normal condition whereas the plants of H. 77/30 appeared unaffected by the divisioning and potting process.

The plants of H. 77/30 Aberystwyth were marked A,

PHOTOGRAPH

PHOTOGRAPH

GREENHOUSE EMPLOYED FOR THE EXPERIMENTS ON THE GROWTH OF TIMOTHY SEED

representing the clonal type and the plants of H. 62/26 were marked B. Each original plant was marked 1, 2 or 3 and the subdivisions of these plants were lettered a, b, c, or d. The plants were watered twice every day, once in the morning and once in the evening. On 8th December the plants were removed from the portico into an open cold frame.

On 11th January 1951, when the spikes were almost ready to flower, the plants were transfered to a pollen-proof greenhouse with an earth floor, (See photograph on page 13). The next day the greenhouse was given two coats of white-wash in order to reduce the heat inside the greenhouse. Felt covers were placed over the three ventilating windows in order to prevent outside pollen from entering. A fan with four propellers (prongs) was situated in the side of the greenhouse thus helping to reduce the temperature within the greenhouse, and a felt cover was placed over the fan ventilating exit when the fan was not being operated. The door into the greenhouse was kept shut and locked.

The dimensions of the ventilating windows were as follows. One large window near the top of the wooden side of the greenhouse was 2' 2" in height and 5' 10" in length with a 4-inch wooden partition in the centre. The two ventilating windows at the bottom of the opposite side of the greenhouse were each 1' 8" in height and 2' 9" in length. The ventilating exit, which contained the fan, had a diameter of 1' $4\frac{1}{2}$" and was

situated between the two small windows.

On 16th January four spikes of Clone A had florets which were in flower. Two of these spikes had one floret with three stamens extended, one spike had two florets and one spike had four florets with stamens extended. On the same day plant A 1 b and B 1 b were taken to the seed testing laboratory at Palmerston North where floret counts were later carried out. The plants of Clone A were further advanced in development than were those of Clone B, the latter being unsatisfactory in the development of spikes.

The spikes of each plant of Clone B were then placed in glazed semi-transparent glassine bags, cotton wool being bound around the stem below the spikes and string tied around the base of the glassine bag and against the cotton wool. In this manner the transfer of pollen between the plants of Clone A and Clone B was prevented.

The intention had been to cross-pollinate the two clones and to carry out separate tests using the seed formed on each clone. However, as a result of the unsatisfactory development of Clone B, it was only possible to carry out the tests with Clone A as the maternal clone, since it was correctly anticipated that there would be insufficient seed formed on the plants of Clone B with which to carry out tests. The spikes of Clone B were used merely as the paternal parent when cross-pollination was undertaken.

M E T H O D S

Flowering and Seed Setting

As mentioned above, two plants in pots labelled A 1 b and B 1 b were handed over to the Seed Testing Station at Palmerston North so that an investigation into the flowering and seed setting habits could be undertaken. It was impossible for the writer to undertake these investigations as well as the weight growth studies since they both had to be carried out at the same time. The officials* at the Seed Testing Station therefore kindly undertook to obtain the information required.

The two pots were kept in the greenhouse on the roof of the Seed Testing Station. Plant A 1 b had three heads tagged, each head being divided into four equal portions designated the Top, Upper Middle, Lower Middle and Lower. Two heads were similarly treated on plant B 1 b. The heads were examined daily from the start of flowering, the anthers being counted and removed both in the morning and the afternoon. When ripe, all the tagged heads were harvested and counts were made of the number of florets on the head and the

* Acknowledgement and appreciation is hereby extended to Misses M. Cowan, D. Matthews and J. Wharton.

number of seeds set.

Daily observations were recorded on the weather at the Seed Testing Station, (see Table H on page 66), and on the temperature and humidity at Grasslands Division, (see Tables I and J on pages 67 and 68).

Self-Sterility

In order to determine the amount of self-fertility occurring within Clone A the plants of this clone were selfed and crossed among themselves. Thus one plant from A 1, A 2 and A 3 was selfed, and one plant from each of them was crossed with one plant of another original plant. The procedure adopted was to bag two spikes of each plant on the 17th January and to count the number of seeds present when the glassine bags were removed on the 3rd April. With the selfed spikes two spikes of one plant were enclosed in a glassine bag, whereas with the crossed spikes two heads from each of two different clonal plants were enclosed in a bag.

At the same time as the flowering and seed setting tests were being carried out, two heads on plant A 1 b and one head on plant B 1 b were tagged at the Seed Testing Station for self-sterility tests. They were each covered with glassine bags and when ripe, the heads were harvested and the seeds and the number of florets per head counted.

Growth

(a) Pollination

In order to obtain an accurate analysis of seed growth it was necessary to cross pollinate on one specific day. The timothy florets flowered at approximately 8 a.m., few florets flowering after 10 a.m. A count was not made at Grasslands Division to substanciate this statement which was based on observation alone. However, this statement was supported by the investigations on the two plants under observation at the Seed Testing Station (See Table I on page 25).

From the above remarks, it can be seen that the majority of seeds would start their growth, following pollination, at the same time even if stray pollen remained viable in the greenhouse after the main pollination was undertaken in the morning.

Thus on the morning of the 29th January, which was anticipated to be the day when the maximum flowering would occur, the glassine bags were removed from the plants of Clone B and the pollen from the spikes of these plants was shaken over the spikes of Clone A.

Since it had been considered that there would be insufficient pollen from Clone B to fertilize satisfactorily the florets of Clone A, spikes from the other plants of Clone B, which were outside in the plot, had been cut two days previous to the date when pollination was carried out. These heads had been kept in another greenhouse in a glass jar full of water. This procedure avoided the chance of pollen other than that of Clone B being introduced onto Clone A since pollen of other plants adhering to the spikes of Clone B, when they were removed from plot, would die within the two days of their being removed.

On 29th January, the spikes of Clone B were removed from the other greenhouse and the pollen from these spikes as well as that of the plants of Clone B which had been grown in the greenhouse, were shaken over the heads of the plants of Clone A.

(b) Dissection and Weighing

Prior to cross-pollinating on 29th January three spikes were removed for the dissection and weighing of the unfertilized ovules. One thousand ovules were dissected, weighed, oven dried and weighed again. Only one of the thousand was found to have developed into a seed. Thus there was ~~approximately~~ .1% self-fertility, which was a similar

percentage to those obtained later in the self-fertility trials.

The ovules were dissected under a Reichert microscope at a magnification of 20X. Ten ovules were dissected at a time and were then placed in an aluminium dish and a metal cover placed over the dish in order to minimise the amount of evaporation occurring. After fifty ovules had been dissected and placed in the dish in this manner, the dish with fifty ovules in it was weighed on a Sartorius weighing scale. By subtracting the weight of the dish, which had previously been determined, from the total weight, the weight of the fifty ovules was obtained. This procedure was repeated. If an interval between dissections occurred the dish with its contents was placed with its lid off in a dessicator so as to evaporate ~~off~~ the moisture in the ovules. When dissection was renewed the dish was removed from the dessicator and shortly afterwards re-weighed previous to the introduction of a fresh batch of ovules into the dish.

After one thousand ovules had been dissected and weighed, the dish with the thousand seeds was placed in a vaccuum oven for five hours at 65°C in order to evaporate off the moisture in the ovules. The dish was then removed from the oven and placed in a dessicator for twenty minutes until it had cooled to room temperature. It was then removed and weighed. The ovules were immediately removed after the weighing and the dish was weighed with its lid. The difference

in weight between the dish, lid and ovules, and the dish and lid, gave the total dry weight of the thousand ovules.

A similar procedure was undertaken every two days thereafter the only alteration being that five hundred seeds were dissected and weighed two days and four days after pollination and that from six to sixty days two hundred and fifty seeds were dissected every two days. The reason for using the large number of ovules and seeds in the earlier stages of growth was to obtain a weight sufficient to obviate errors induced by small weights.

Germination

Every two days, that the weight growth tests were made, seed was retained for use in a germination test to be carried on later. Although the weight growth test was started on January 29th, the germination test did not start until April 9th at 5 P.M.

Fifty seeds were prepared in envelopes representing each two days from January 29th to March 30th. A trial, shown in Table F on page 64, was commenced on April 3rd. When it was apparent, after two days of the trial, that almost complete

germination was being obtained, on April 8th the main test was commenced.

Thirty-one petrie dishes were collected and unprocessed cotton wool was placed in the bottom section of the dishes. A filter paper was placed on top of the cotton wool. On each filter paper was written the number of days from pollination to harvesting of the particular seeds to be placed in the petrie dish. Distilled water was then poured into each dish and the cotton wool and filter paper allowed to absorb the moisture in such a manner that there was only a small surplus of water in the dish.

When the above procedure was completed the fifty seeds for each of the thirty-one two day selections were placed in their individual petrie dishes on the filter papers as rapidly as possible in order that they would each start absorbing the water at the same time. The covers to the petrie dishes were then placed on the petrie dishes and over the seeds.

The petrie dishes were then placed in one glass-topped incubator, which was kept at 20°Centigrade and at a hundred percent humidity. The dishes lay on glass slabs which ran across a tank filled with water.

At twenty-four hour intervals, after placing the seeds in the germinator, a count was made of the number of seeds

germinating in each dish. When the plumule and radicle was visible to the naked eye the seed was considered to have germinated.

Effect of Chilling On Dormant Seeds

Those seeds that did not germinate after forty-three days in the incubator were subjected to seven periods of chilling. The first three, and the sixth and seventh, chilling treatments consisted of placing the ungerminated seeds in the refrigerator at 3°C for seven days and then replacing them in the incubator at 20°C for varying periods of time. Those seeds that did not germinate after the first chilling treatment were later subjected to a further treatment of chilling followed by incubation. The fourth chilling treatment was continued for nine days and the fifth for sixty-six days.

Seedling Growth From Chilled Seeds

The seedlings developing from the chilled seeds were kept in petrie dishes in the incubator until their maximum growth had been obtained. They were then removed, and measured.

TABLE I

TABLE I

FLOWERING OF TIMOTHY

Occurence of Blooming of Florets (or blooming each day) in different segments of the head throughout the flowering period.

Plant Number	Tagging colour of head	Segment of Head	Number of Florets Blooming on Specified Dates 19/1	20/1	21/1 (a)(b)	22/1	23/1	24/1	25/1	26/1	27/1	28/1	29/1	30/1	31/1	1/2	2/2	3/2	4/2	5/2	6/2	7/2	8/2	Total Florets Blooming per Segment	Number of days to complete Flowering
A1b	Green	Top	3	-	42+2	23+3	-	1	62+5	-	1	-	17	-	-	-	-	-	-	-	-	-	-	159	11
		Upper Middle	8	-	41+3	39+19	1	3	71+21	4	-	1	20	1	4	1	2	1	-	-	-	-	-	240	16
		Lower Middle	4	-	75	39+20	1	4	80+31	7	3	1	47+1	4	2+2	1	5	-	-	-	-	-	-	327	15
		Lower	1	-	49	26+16	2	3	61+21	6	1	-	28+3	1	3	1	2	-	-	-	-	-	-	224	15
		TOTAL	16	-	212	185	4	11	352	17	5	2	116	6	11	3	9	1	-	-	-	-	-	950	
	Brown	Top			23+1	34+1	1	1	59+9	1	1	-	39	-	1+1	-	-	-	-	-	-	-	-	172	11
		Upper Middle			36+1	41+8	-	1	88+29	3	1	-	32+1	-	3	3	2	-	-	-	-	-	-	249	13
		Lower Middle			34	36+11	-	2	67+21	2	-	-	14+1	1	3+2	3	3	-	-	-	-	-	-	200	13
		Lower			38	40+12	-	7	84+30	6	3+1	-	30+1	-	8+2	1	2	-	-	-	-	-	-	265	13
		TOTAL			133	183	1	11	387	12	6	-	118	1	20	7	7	-	-	-	-	-	-	886	
	Blue	Top	-	-	16	23	-	-	53+10	2	2	-	50	-	-	-	-	-	-	-	-	-	-	156	9
		Upper Middle	1	-	22	30+7	-	-	88+29	2	2	-	54+6	7	5+4	1	3	-	-	-	-	-	-	261	15
		Lower Middle	-	-	4	14+8	1+1	-	45+21	5	-	-	35+1	1	1+2	-	4	-	-	-	-	-	-	143	13
		Lower	-	-	1	8+7	1	-	32+12	1	-	-	17	1	1	-	2	-	-	-	-	-	-	83	13
		TOTAL	1	-	43	97	3	-	290	10	4	-	163	9	13	1	9	-	-	-	-	-	-	643	
B1b	Green	Top			-	-	-	38	-	-	1	170	6	3	-	7	-	-	-	-	-	-	-	225	9
		Upper Middle			-	-	-	91+18	1	1	1	150	11	3	-	14	1	-	-	-	-	-	-	291	10
		Lower Middle			2	9+1	17	90+27	3	2	1	94	12	3	-	20	-	2	-	-	-	-	-	283	14
		Lower			-	-	-	36+12	5	-	5	71+1	14	4	-	6	3	-	-	-	-	-	-	157	10
		TOTAL			2	10	17	312	9	3	8	486	43	13	-	47	4	2	-	-	-	-	-	956	
	Blue	Top					-	-	-	-	-	-	36	25	5+4	-	22	103	10	-	-	-	-	205	7
		Upper Middle					1	-	28	-	-	-	70	29	19	-	21	80	9	1	-	-	1	259	17
		Lower Middle					-	-	23+1	-	-	1	80+1	37	5	-	11	52	3	3	-	-	-	217	12
		Lower					-	-	4	-	-	-	46+1	23	31	-	12	57	17	22	6	5	-	224	14
		TOTAL					1	-	56	-	-	1	234	114	64	-	66	292	39	26	6	5	1	905	

(a) Count made at 8.a.m. (b) Count made at 4.p.m.

R E S U L T S A N D D I S C U S S I O N

Flowering and Seed Setting

(a) Flowering

The results obtained from the flowering counts are shown in Table I on page 25. Figures, such as 23+1 on the 21/1, represent the morning count plus the afternoon count. Where only one figure is shown it represents a morning count. The morning counts were undertaken at 8 a.m. and the afternoon counts at 4 p.m. The counts showed that the maximum flowering took place in the early morning.

It can be seen that flowering was abundant on certain days, but that between these popular days there were days when practically no flowering occurred. These popular flowering days were the same for each of the three heads of plant A 1 b under investigation. However, by referring to the weather records shown in Tables H and I on pages 66 and 67 there appears to be no readily noticeable correlation between maximum flowering and maximum temperature.

The popular flowering days of the heads of plant B 1 b under investigation did not occur on the same days as did those of plant A 1 b. An exception to this occurred on 29th January when there were numerous flowers on the three A 1 b heads and also on one of the two B 1 b heads. The two heads of plant B 1 b had different popular flowering days between each other throughout their periods of flowering.

Flowering continued on all four segments of all the tagged heads of both plants for an average of twelve and a half days. The minimum and maximum duration of flowering for any segment of any of the heads observed was 9 and 16 days for plant A 1 b and 7 and 17 days for plant B 1 b. However, on every tagged head, the Top segment had the shortest duration of flowering, the minimum and maximum being 7 and 11 days.

The main purpose of the flowering test was to determine if there was any difference between the time of flowering of the different parts of the timothy head. The Analyses of Variance* of the flowering counts for January 21, 22, 25 and 29, 1951 are shown in Table A on page 58 and 59, the differences between the flowering parts of the head not being significant on any of the dates in question.

* In all analyses of variance the following abbreviations are used:
N.S. = not significant at the 5% level
* = significant at the 5% level but not at the 1% level.
** = significant at the 1% level.

TABLE II

TABLE II

FLOWERING AND SEED SETTING OF TIMOTHY

Showing the number of florets in each segment of the head and the proportion of florets blooming and setting seed.

Plant Number	Tagging Colour of Head	Segment of Head	Number of Florets	Number Blooming	Number Setting Seed	Percentage Florets Blooming	Percentage Florets Setting Seed	Number of Seeds as a Percentage of Florets Blooming
A 1b	Green	Top	450	159	95	35	21	60
		Upper Middle	408	240	106	58	26	44
		Lower Middle	476	327	147	69	31	45
		Lower	508	224	80	44	16	36
		TOTAL	1842	950	428			
	Brown	Top	436	172	91	39	21	53
		Upper Middle	508	249	117	49	23	47
		Lower Middle	410	200	87	49	21	43
		Lower	547	265	88	48	16	33
		TOTAL	1901	886	383			
	Blue	Top	340	156	70	46	21	45
		Upper Middle	457	261	96	57	21	37
		Lower Middle	383	143	38	37	10	27
		Lower	316	83	29	26	9	35
		TOTAL	1496	643	233			
	AVERAGE		1746	826	348	47°3	19°9	42°1
B 1b	Green	Top	628	225	69	36	11	31
		Upper Middle	765	291	148	38	19	51
		Lower Middle	551	283	134	51	24	47
		Lower	707	157	79	22	11	50
		TOTAL	2651	956	430			
	Blue	Top	396	205	48	52	12	23
		Upper Middle	382	259	85	68	22	33
		Lower Middle	356	217	94	61	26	43
		Lower	470	224	74	48	16	33
		TOTAL	1604	905	301			
	AVERAGE		2127	930	365	43°7	17°2	39°3
AVERAGE FOR A1b + B1b			1899	868	355	45·7	18·7	40·9

(b) Seed Setting

When the tagged heads were ripe they were each harvested in four separate divisions, namely Top, Upper Middle, Lower Middle and Lower. Counts were then made of the number of florets and the number of seeds set in each division of each head. The results of these counts are shown in Table II on page 28. Included in this table is the total number of florets seen to flower during the flowering test. The percentages of flowers+ to florets, seed to florets and seed to flowers is also shown. The mal-formed and incomplete florets at the base and at the top of the head were not included in the sectioning or in the counting undertaken in these flowering and seed setting tests.

The Analyses of Variance of the percentage seed to florets, flowers to florets and seed to flowers, all for the four positions of the timothy heads, are shown below.

Percentage Seed to Florets for Four Positions of the Timothy Heads

(Percentages transferred to arc $\sin\sqrt{\text{Percentage}}$)*

Source of Variation	Sums of Squares	Degrees of Freedom	Mean Squares	F	Results
Heads	117.8	4	29.450		
Positions	150.0	3	50.000	3.82	*
Error	157.0	12	13.083		
Total	424.8	19			

+On pages 29-31 "flowers" has been used in place of "florets blooming"

* Snedecor (1948) p.449

The difference between positional means for significance at the 5% level is;

$$2.179\sqrt{\frac{2(13.083)}{5}} = 4.99$$

The means are, (transformed percentages),

Upper Middle	28.2
Lower Middle	27.8
Top	24.0
Lower	21.6

Hence, the Lower part of the heads gives significantly less seed set per floret than either the Upper Middle or the Lower Middle but does not differ significantly from the seed set of the Top.

Percentage Flowers to Florets for Four Positions of the Timothy Heads

(Percentages transferred to arc $\sin\sqrt{\text{Percentage}}$)

Source of Variation	Sums of Squares	Degrees of Freedom	Mean Squares	F	Results
Heads	366.70	4	91.67		
Positions	361.75	3	120.58	4.35	*
Errors	332.50	12	27.71		
Total	1060.95	19			

The Positional means are (transformed data),

Top	40.2
Upper Middle	47.4
Lower Middle	47.0
Lower	37.6

The difference between pairs of these required for significance at the 5% level is;

$$d = 2.179\sqrt{\frac{2\ (27.708)}{5}} = 7.25$$

Hence, the percentages of flowers to florets in the two middle sections of the head are significantly greater than the percentage in the Lower section, and almost significantly greater than in the Top section on the head.

Percentage Seed to Flowers for Four Positions of the Timothy Heads

(Percentages transferred to arc $\sin\sqrt{\text{Percentage}}$)

Source of Variation	Sums of Squares	Degrees of Freedom	Mean Squares	F	Results
Heads	205.7	4	51.42		
Positions	28.2	3	9.40	-	N.S.
Errors	380.3	12	31.69		
Total	614.2	19			

Hence, there is no significant difference between the mean percentages of seed to flowers for the four positions on the heads.

It can be seen from Table II on page 28 that the total average of the florets on the five timothy heads was 1899, of which 868 flowered and 355 set seed. Thus the total percentages of flowers to florets, seed to florets and seed to flowers for plant A 1 b plus plant B 1 b was almost 46, 19 and 41 percent respectively.

T A B L E III

T A B L E IV

T A B L E V

TABLE III

Self-Sterility of Timothy - Trial 1

Seed setting resulting from self-Pollination.

Plant	Number of Heads	Number of Seeds
A1c	2	2
A2a	2	1
A3b	2	0
TOTALS	6	3

TABLE IV

Self-Sterility of Timothy - Trial 2

Seed setting resulting from Crossing Plants of the same Clone.

Plant			Number of Heads	Number of Seeds
A1c	x	A3c	2 x 2	0
A1d	x	A2a	2 x 2	0
A2b	x	A3b	2 x 2	0
TOTALS			12	0

TABLE V

Self-Sterility of Timothy - Trial 3

Seed setting resulting from self-pollination.

Plant	Number of Heads	Number of Florets	No. of Seeds	Seeds set as a percentage of Florets
A1b	1	925	0	0
A1b	1	1212	2	·165
B1b	1	1113	1	·09
TOTALS	3	3250	3	·09

Self-Sterility

The individual plants used in the selfing and crossing trials of Clone A and the results obtained are shown in Tables III and IV on page 32.

Only three seeds were obtained from the six heads used in the self-pollination trial and no seeds were procured from any of the twelve heads used in the trial on cross-pollination within clonal plants.

Table V on page 32 shows that, from the three heads tagged at the Seed Testing Station, three seeds were obtained and that this represented a seed set of .09 percent in relation to the florets present on the heads.

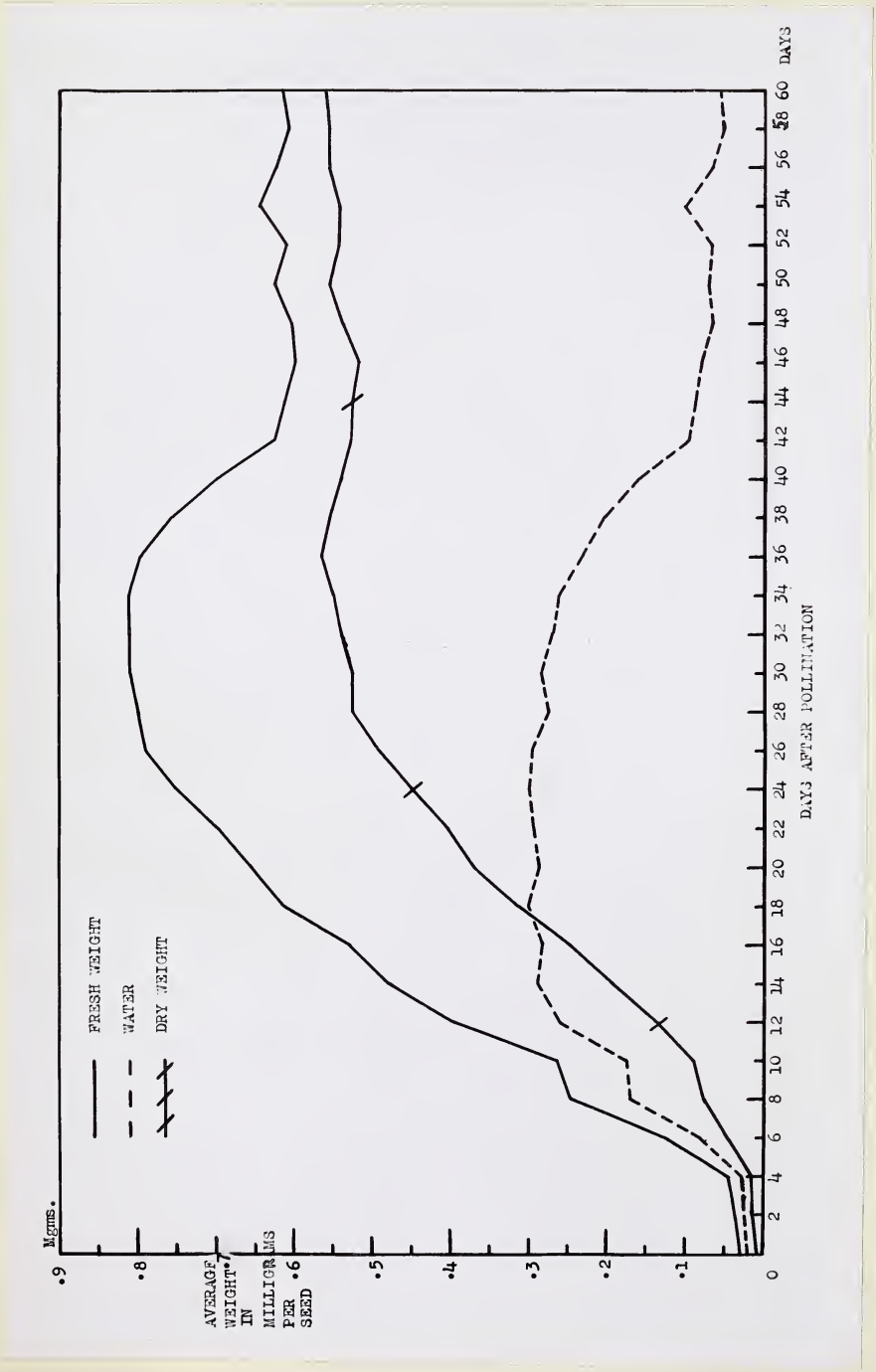
It is thus assumed that any self-fertility likely to occur within Clone A would not affect to any significant extent the results obtained from the Growth or the Germination Experiment.

FIGURE A

F I G U R E A

GROWTH OF TIMOTHY SEED

Changes in Fresh Weight, Dry Weight and Water Content from Measurements on Samples Harvested at Two Day Intervals from Pollination.



Growth

The lists of the total and the average fresh weight, dry weight and water content and the percentage composition of dry matter and water are shown in Table C on page 62. In Table B on page 60 is also shown the fresh weights for each fifty seeds. Figure A on page 34 portrays the relative fresh weight, dry weight and water content of the seeds during their growth.

(a) Fresh Weight

The over-all seed weight increases comparatively slowly until the sixth day, when an almost three fold increase over the fourth day occurs. After this the weight increase is rapid but in the form of gradually decreasing increments. On the thirty-fourth day the peak is reached at an average of .8128 milligrams per seed. From this day onward the fresh weight decreases in the form of an increasingly steep curve until the forty-second day is reached when a flattening of the curve occurs. On the forty-sixth day the minimum mature fresh seed weight is reached.

(b) Dry Weight

The dry weight increases to the maximum average of .566 milligrams thirty-six days after pollination. After this time the weight remains approximately constant at .55 milligrams, although an inexplainable decrease occurs to .5188 milligrams on the forty-sixth day.

(c) Water Content

The water content of the seeds is obtained by subtracting the dry weight from the fresh weight. Thus the two curves described above have a direct bearing on the water content curve.

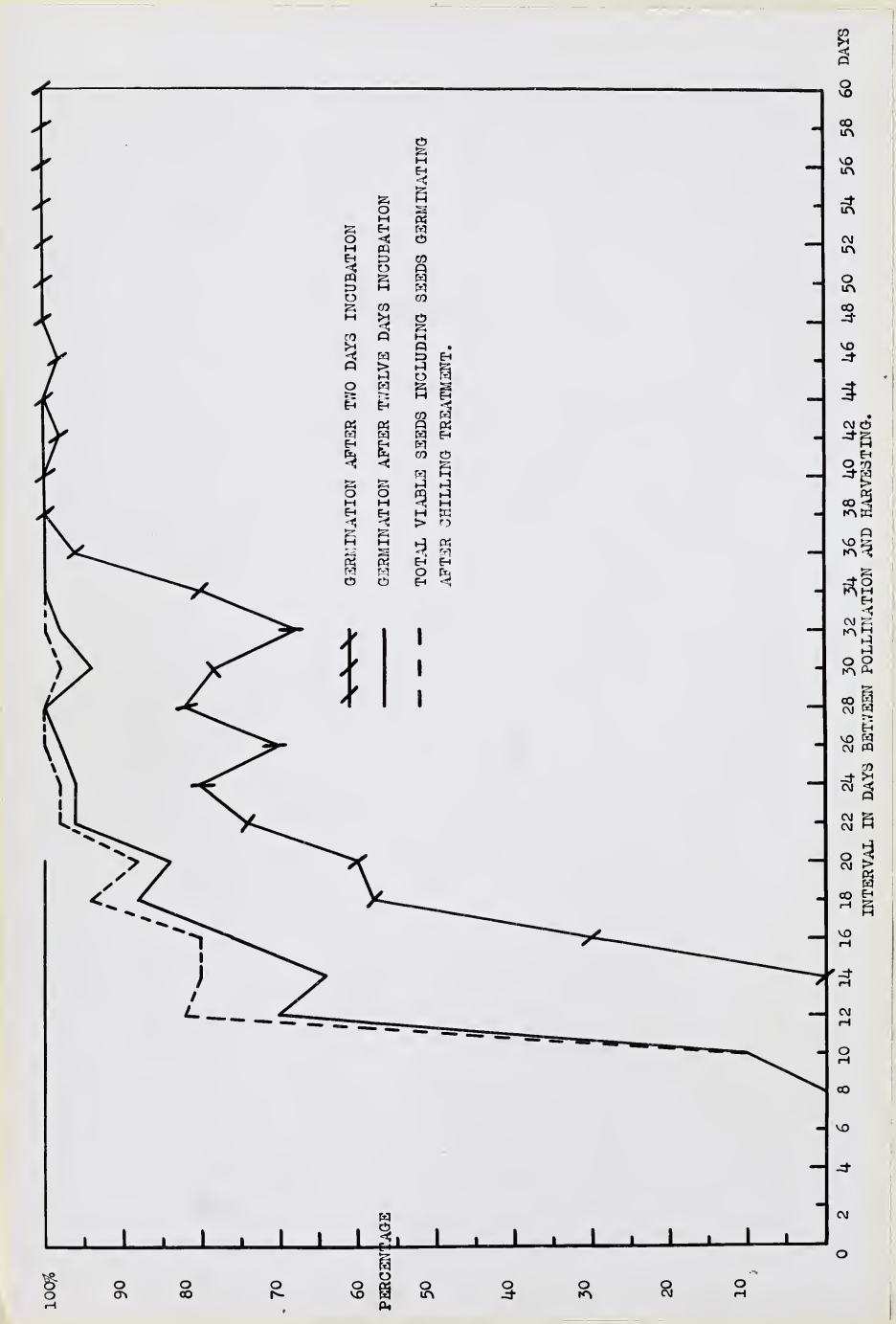
The weight of the water content increases slowly until the fourth day but increases considerably on the sixth and on the eighth day. On the tenth day there is an inexplainable lag in the water increase. On the fourteenth day the approximate peak is reached at an average of .287 milligrams per seed and the curve levels off. An absolute peak occurs on the twenty-fourth day at .3016 milligrams per seed. From the twenty-fourth day a gradually increasingly steep descent occurs until the forty-second day when the curve flattens out, the approximate minimum being obtained after forty-eight days. An inexplainable jump occurs on the fifty-fourth day.

FIGURE B

F I G U R E B

GERMINATION OF TIMOTHY SEED

Influence of Stage of Seed Growth at Harvesting on the Germinating Capacity, Speed of Germination and Incidence of Dormancy.



Germination

The first seeds to germinate was obtained from seeds removed ten days after pollination (see Figure B on page 37 and Tables E and F on pages 63 and 64). This seed was slow in germinating as the earliest germinated only after four days in the incubator and developed into weak seedlings. It was observed that the allowance of an increased number of days from pollination to the harvesting of the seed resulted in a stronger seedling. This is born out by measurements made later on the length of seedlings germinating after the application of a period or periods of chilling, (See Table G on page 65).

The germination after twelve days in the incubator jumped from ten percent for seed removed ten days after pollination to seventy percent for seed removed twelve days after pollination. Seed removed eighteen days after pollination gave eighty-six percent germination after five days in the incubator.

Seed harvested twenty-two days or more after pollination never gave a lower germination percentage than ninety-four after six days in the incubator. Only one of these seeds germinated between the eighth day of incubation, and the commencement of the chilling experiment.

No seeds germinated within twenty-four hours of being placed in the incubator, but seed removed sixteen days after pollination gave thirty percent germination after two days. Seed removed twenty-two days after pollination gave seventy-four percent germination after two days incubation.

Seed allowed less than thirty-six days between pollination and harvest gave a maximum germination of eighty-two percent after two days in the incubator. However, an interval of thirty-six days gave ninety-six percent germination after two days incubation and only two seeds removed at periods greater than this did not germinate within two days.

Columns 3 and 4, in Table F on page 64, show the seeds that germinated toward the end of the forty-three days of incubation. These seeds were all very feeble in their growth.

TABLE VI

TABLE VI

EFFECT OF CHILLING ON DORMANT TIMOTHY SEEDS

Seeds chilled at 3°c. and subsequently Incubated at 20°c.

Number of days from Pollination to Harvest	Total firm seeds ungerminated before chilling	First Chilling Treatment May 23 to May 30 No. of seeds germinating after chilling in specified No. of days Incubation					Second Chilling Treatment June 20 to June 27 No. of seeds germinating after chilling in specified No. of days Incubation					Fifth Chilling Treatment November 6/51 to January 11/52 No. of seeds germinating after chilling in specified No. of days Incubation				
		1	2	3	4	21	2	3	4	5	31	2	3	4	5	40
0 to 10	0															
12	9			2	3	3		1	1	1 feeble 2	2					
14	8		1	2	4	4			1	2	2		1	1	1	1
16	3												1	1	1	1
18	2		2	2	2	2										
20	2															
22	0															
24	1		1	1	1	1										
26	0															
28	0															
30	2		2	2	2	2										
32	1												1	1	1	1
34 to 60	0															
TOTALS	28					12					4					3
Percentage	100					42·9					14·3					10·7

Third Chilling Treatment July 28 to August 4. No seeds germinated during twenty-four days subsequent incubation.

Fourth chilling treatment August 28 to September 6th. No seeds germinated during two months (until November 6) subsequent incubation.

Sixth Chilling Treatment February 20/52 to February 27. No seeds germinated during eighteen days subsequent incub-ation.

Seventh Chilling Treatment March 16 to March 23. No seeds germinated during twenty days subsequent incubation.

Effect of Chilling on Dormant Seeds

Since no seed removed twenty-two days and longer after pollination gave a lower percentage of germination than ninety-four after six days incubation, the effect of chilling upon the ungerminated seed referred chiefly to seed which had been harvested less than twenty-two days after pollination.

Seed removed on the tenth day after pollination produced only ten percent viable seed either before or after the application of periods of chilling. The unviable seeds were soft and yellow in appearance.

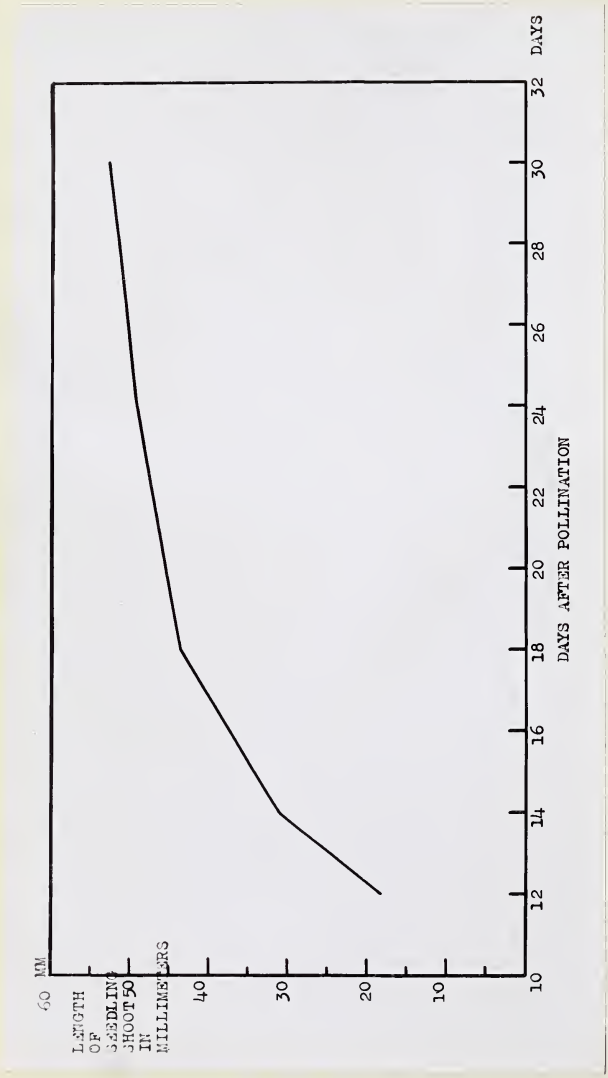
The effect of chilling is shown in Tables VI and F on pages 40 and 64. It can be seen in Table VI, that, of the twenty-eight seeds which remained firm but did not germinate previous to chilling, 42.9 percent became viable after the first period of chilling and that over two-thirds became viable after five periods of chilling. Only one seed that germinated after the chilling treatments remained very feeble. This seed had been removed twelve days after pollination and germinated following the second period of chilling. In Table F, the grand total of seeds germinating shows that at least 80 percent of the seeds removed twelve days or more after pollination eventually germinated.

F I G U R E C

F I G U R E C

GROWTH OF TIMOTHY SEEDLINGS

Influence of the Time of Harvesting of the Seed on the Length of the Seedling shoot.



Seedling Growth From Chilled Seeds

While the number of seedlings used in this measurement test had of necessity to be small, nevertheless, the length of the seedlings for any particular number of days after pollination was in all cases approximately the same. In no instance did the length of a seedling from a particular day's harvest exceed the length of a seedling which had been allowed a longer period to develop after pollination had occurred. (See Figure C and Table G on pages 42 and 65).

The Analyses of Variance, by which shoot lengths at twelve and fourteen days, and fourteen and eighteen days are compared, are shown below. (The data used are the results from the First and Second chilling).

Source of Variation	Sums of Squares	Degrees of Freedom	Mean Squares	F	Result
Between 12 & 14 Days	240	1	240	17.78	**
Error	108	8	13.5		
Total	348	9			

Source of Variation	Sums of Squares	Degrees of Freedom	Mean Squares	F	Result
Between 14 & 18 Days	315.4	1	315.4	19.21	**
Error	98.5	6	16.42		
Total	413.9	7			

The statistical analyses show that for the results of the first two chillings, there was a highly significant difference between the mean lengths of the seedlings from seeds harvested at twelve days and at fourteen days after pollination and also between those harvested at fourteen days and eighteen days.

C O N C L U S I O N

Under natural conditions in the field environmental influences would be considerably different to those existing in a greenhouse, which contained only two different timothy clones. In the greenhouse, pollination was carried out by shaking the heads of the two clones together whereas under field conditions, pollination would take place through the medium of the wind. Many ecological factors, such as temperature, humidity and precipitation, would be such that entirely different results might be obtained under field conditions.

Flowering took place over approximately two weeks within which days of heavy or light flowering occurred. Of the total number of florets, less than half flowered, and of the flowering florets, approximately forty percent set seed. The timothy plants under observation showed almost complete self-sterility.

The growth of timothy seed until the fourteenth day was mainly the result of moisture increase. From this interval of time until the thirty-sixth day, the dry weight continued to increase whereas the increase in water content slowed down to such an extent that by the twenty-eighth day it had started to drop. Both the increase and decrease of the water content was rapid whereas the dry weight increased more

slowly but for a longer period. After the thirty-sixth day the dry weight remained approximately stationary. However, as a percentage of the fresh weight, the dry weight increased from approximately thirty-four percent during the first twelve days of growth until it reached over ninety-one percent fifty-eight days after pollination.

Since all the seeds tested were placed in the incubator at the same time, the seeds harvested in the earlier stages of growth had up to two months longer than the last seeds harvested to overcome any after-harvest dormancy which they might have possessed. This could affect the results of germination capacity obtained from the earlier harvested seeds.

It would appear that in order to obtain high germination, timothy seed should be allowed twenty-two days from pollination to harvest. However, in an emergency harvesting could be carried out only twelve days after pollination and this might result in as high as seventy percent germination under favourable conditions. Eight days of incubation appears to be the period that is required to test the germinating capacity of seed harvested twenty-two days or longer after pollination, although six days incubation might be a sufficient duration. The incidence of dormancy after twelve days incubation was very low, occurring

mainly in seed harvested twenty or less days after pollination.

The test on the chilling of timothy seed was not extensive since it included only those few seeds that had not germinated during forty-three days incubation, but it is apparent that immature timothy seed is affected in its germinating capacity by chilling.

Although the seeds germinating toward the end of the forty-three days incubation were very feeble in their growth, all except one that germinated after one or more periods of chilling were normal and vigorous in their growth.

It had been observed during the main germination test, that there was a steady increase in the initial vitality of the seedlings corresponding to an increase in the times allowed for seed growth after pollination. This was substantiated by the observations on the seedling growth of chilled timothy seed. The viability and initial vitality of the timothy seed during its period of growth after pollination appear to be correlated.

It is probable that seed size, speed of germination and seedling vigour will all improve as growth progresses up to the stage of full maturity, which, in these experiments, appeared to occur on the thirty-sixth day.

S U M M A R Y

1. Records were obtained of the order and duration of flowering of florets on different segments of the timothy flower heads, of the number of florets per head and per segment and of the number of seeds set per head and per segment.

The differences between the time of flowering of segments of the timothy heads were not significant. The Lower part of the head gave significantly less seed set per floret than either the Upper Middle or the Lower Middle but did not differ significantly from the seed set of the Top segment. The percentages of flowers to florets in the two Middle segments of the head were significantly greater than the percentage in the Lower segment, and almost significantly greater than in the Top segment. There was no significant difference between the mean percentages of seed to flowers for the four positions on the heads. Of the total florets present, less than half flowered and of the flowering florets, approximately forty percent set seed.

2. Three trials were carried out to determine the degree of self-sterility occurring within clonal plants of

timothy. These self-sterility trials showed that less than .1 percent of the timothy florets set seed following selfing.

3. The increase in weight of the timothy seeds following fertilization was determined. The fertilization was undertaken manually in bulk on one specific day and seed was removed every two days thereafter at which time the seeds were dissected and weighed to obtain their fresh and dry weights and thereby their water content.

The growth of the seed increased mainly by means of moisture increase until the fourteenth day when the water content levelled off. After this period, the dry weight content continued to increase and it was this factor which was responsible for an increase in the over-all fresh weight. The water content started to drop on the twenty-eighth day but the increase in the dry weight was sufficient to maintain an increase in the fresh weight until the thirty-sixth day when the decreasing water content lowered the fresh weight. It was on this day that the dry weight had reached its peak and henceforth remained at this approximate weight.

4. Seed heads were retained every two days during the growth experiment for use in a later experiment that was undertaken to determine the germination capacity of seed at two-day intervals during growth.

The average dry weight of seeds harvested twenty-two days after pollination was found to be .404 milligrams whereas the maximum dry weight, on the thirty-sixth day after pollination, was .566 milligrams. This represents an increase of forty percent. However, on the twenty-second day after pollination and after only five days in the incubator, ninety-six percent of the seeds germinated. Thus a very high percentage of the seeds germinated rapidly, although they had not remained on the timothy plant for sufficient time to obtain their maximum dry weight.

5. The timothy seeds that did not germinate within forty-three days were subjected to periods of seven days of chilling, in order to determine the effect of chilling upon germination. In the intervals between these chilling periods the seeds were replaced in the incubator.

Of the seeds that had been removed twelve days and more after pollination, and had not yet germinated after forty-three days in the incubator, thirty-three were soft unviable seeds and twenty-eight were firm seeds. From the latter, over two-thirds germinated after one to five periods of chilling at 3°C.

6. The timothy seedlings developing from chilled seeds were measured at their maximum growth in the incubator in order to ascertain if there was any difference in the initial

vitality of seedlings developing from seed having different periods of growth before harvesting.

An increase in the length of seedling was found to correspond with an increase in the length of time between pollination and harvesting.

A C K N O W L E D G E M E N T S

The information presented in this thesis was obtained from experiments carried out in the early months of 1951 at the Grasslands Division of the Department of Scientific and Industrial Research, Palmerston North, New Zealand. E. Bruce Levy, until recently, Director of Grasslands Division, D.S.I.R. and L. Corkill, Senior Plant Breeder, kindly allocated a greenhouse at Grasslands Division, and Dr. J. Melville, present Director of Grasslands Division, readily made available the facilities at the Plant Chemistry Laboratory.

The topic was undertaken after consultation with E.O.C. Hyde, Senior Seed Research Officer, Plant Chemistry Laboratory, D.S.I.R., whose suggestions, criticisms and encouragement are deeply appreciated by the writer. The advice received from A.C. Glenday on the statistical analyses and the photography carried out by Miss D.M.P. Oakley are both acknowledged with appreciation.

Misses M. Cowan, D. Matthews and J. Wharton of the Seed Testing Station, Palmerston North, with the permission of A. V. Lithgow, undertook to record the flowering and seed setting of timothy and the writer hereby extends his thanks.

The interest shown by Dr. L. P. V. Johnson, Professor of Genetics and Plant Breeding at the University of Alberta and by W. A. Jacques and G. S. Robinson of the Field Husbandry Department, Massey Agricultural College, Palmerston North, is appreciated.

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A P P E N D I X

A P P E N D I X

TABLE A

ANALYSES OF VARIANCE OF THE POSITION OF FLOWERING OF TIMOTHY

Position of Flowering of Timothy on January 21, 1951

(Data transformed to Square Roots)

Source of Variation	Sums of Squares	Degrees of Freedom	Mean Squares	F	Result
Heads	4.350	1	4.350		
Positions	2.300	3	.766	1.40	N.S.
Error	1.649	3	.549		
Total	8.299	7			

Position of Flowering of Timothy on January 22, 1952

(Data transformed to Square Roots)

Source of Variation	Sums of Squares	Degrees of Freedom	Mean Squares	F	Result
Heads	9.252	2	4.626		
Positions	4.497	3	1.499	2.64	N.S.
Error	3.408	6	.568		
Total	17.157	11			

TABLE A (continued)

Position of Flowering of Timothy on January 25, 1952

(Data not transformed to Square Roots)

Source of Variation	Sums of Squares	Degrees of Freedom	Mean Squares	F	Results
Heads	1207.50	2	603.750		
Positions	2864.92	3	954.973	2.13	N.S.
Error	2691.83	6	448.638		
Total	6764.25	11			

Position of Flowering of Timothy on January 29, 1952

(Data transformed to Square Roots)

Source of Variation	Sums of Squares	Degrees of Freedom	Mean Squares	F	Results
Heads	2.232	2	1.116		
Positions	1.270	3	.424	-	N.S.
Error	13.975	6	2.329		
Total	17.477	11			

GROWTH OF TIMOTHY SEED

Weights in Milligrams of Samples from Individual Plants, Harvested at Two Day Intervals After Pollination.

Da Af Po at	Days After Polli-nation	Plant	No. of Ovules or Seeds	Fresh Weight	Total Dry Weight	Total Water
2	0	A1d	100	·0034		
		A1d	100	·0024		
		A1d	100	·0036		
		A1d	100	·0030		
		A3a	100	·0024		
		A3a	100	·0026		
3		A3a	100	·0026		
		A2d	100	·0032		
		A2d	100	·0033		
		A2d	100	·0022		
	TOTAL		1000	·0287	·0092	·0195
	2	A2d	50	·0019		
		A2d	50	·0018		
3		A2d	50	·0020		
		A2d	50	·0017		
		A2d	50	·0016		
		A2c	50	·0017		
		A2c	50	·0023		
		A2c	50	·0018		
		A3d	50	·0019		
		A3d	50	·0016		
3	TOTAL		500	·0183	·0068	·0115
	4	A2d	50	·0021		
		A2d	50	·0026		
		A2d	50	·0022		
		A2d	50	·0024		
		A2d	50	·0017		
		A2d	50	·0017		
3		A1d	50	·0027		
		A1d	50	·0016		
		A1d	50	·0025		
		A1d	50	·0028		
	TOTAL		500	·0223	·0083	·0140
	6	A1d	50	·0052		
		A3d	50	·0070		
		A3d	50	·0066		
		A2b	50	·0074		
		A2b	50	·0060		
	TOTAL		250	·0312	·0209	·0203
	8	A1d	50	·0134		
		A3d	50	·0130		
		A3d	50	·0125		
		A2b	50	·0118		
		A2b	50	·0104		
	TOTAL		250	·0611	·0187	·0424
	10	A2d	50	·0132		
		A2d	50	·0120		
		A2d	50	·0133		
		A3c	50	·0138		
		A3c	50	·0133		
	TOTAL		250	·0656	·0218	·0438
	12	A3d	50	·0203		
		A3d	50	·0211		
		A3d	50	·0187		
		A1d	50	·0192		
		A1d	50	·0191		
	TOTAL		250	·0984	·0336	·0648
	14	A1c	50	·0249		
		A3d	50	·0240		
		A2c	50	·0239		
		A2c	50	·0230		
		A2c	50	·0241		
	TOTAL		250	·1199	·0481	·0718
	16	A1d	50	·0251		
		A1d	50	·0255		
		A2d	50	·0296		
		A2d	50	·0250		
		A3d	50	·0273		
	TOTAL		250	·1325	·0620	·0705
	18	A1d	50	·0305		
		A1d	50	·0309		
		A2d	50	·0302		
		A3d	50	·0309		
		A3d	50	·0311		
	TOTAL		250	·1536	·0787	·0749
	20	A1d	50	·0333		
		A1d 28 / A2d 22		·0327		
		A2d	50	·0328		
		A2d	50	·0321		
		A3a	20	·0331		
		A3c	30			
	TOTAL		250	·1640	·0924	·0716
	22	A3c	50	·0358		
		A3c	50	·0335		
		A1a	50	·0350		
		A1d	50	·0354		
		A3a 25 / A3c 25		·0347		
	TOTAL		250	·1744	·1010	·0734
	24	A1a	50	·0382		
		A1a	50	·0386		
		A2d	50	·0362		
		A3c	50	·0370		
		A1d	50	·0377		
	TOTAL		250	·1877	·1123	·0754
	26	A1a	50	·0390		
		A3c	50	·0413		
		A3d 22 / A3b 28		·0397		
		A3c	50	·0380		
		A2b 25 / A2d 25		·0393		
	TOTAL		250	·1973	·1231	·0742

TABLE B — continued

Days After Pollination	Plant	No. of Ovules or Seeds	Fresh Weight	Total Dry Weight	Total Water
28	A1a	50	·0411		
	A1a	50	·0425		
	A3a	50	·0372		
	A3a	50	·0402		
	A2b	50	·0394		
	TOTAL	250	·2004	·1317	·0687
30	A3a	25			
	A3d	25	·0444		
	A3d	50	·0412		
	A3d	50	·0387		
	A2c	50	·0393		
	A2c	50	·0389		
	TOTAL	250	·2025	·1315	·0710
32	A2c	50	·0416		
	A2c	50	·0413		
	A2c	30			
	A1d	20	·0415		
	A1d	20			
	A1c	30	·0374		
	A1c	50	·0410		
	TOTAL	250	·2028	·1350	·0678
34	A1a	50	·0386		
	A3a	50	·0426		
	A3a	30			
	A2d	20	·0443		
	A1a	50	·0391		
	A2b	50	·0386		
	TOTAL	250	·2032	·1377	·0655
36	A2d	50	·0433		
	A2d	50	·0354		
	A2d	35			
	A3d	15	·0378		
	A3d	50	·0418		
	A3d	23			
	A1a	27	·0413		
	TOTAL	250	·1996	·1415	·0581
38	A3c	50	·0400		
	A1a	50	·0382		
	A1a	25			
	A3c	25	·0382		
	A2c	50	·0366		
	A2d	50	·0367		
	TOTAL	250	·1897	·1388	·0509
40	A3d	50	·0352		
	A3d	50	·0331		
	A3a	50	·0351		
	A2d	50	·0367		
	A2d	50	·0350		
	A2c	50			
	TOTAL	250	·1751	·1348	·0403
42	A1d	50	·0306		
	A1d	32			
	A1a	18	·0320		
	A2c	50	·0325		
	A3a	50	·0311		
	A3a	50	·0301		
	TOTAL	250	·1563	·1319	·0244
44	A1d	50	·0293		
	A1d	50	·0285		
	A1a	50	·0310		
	A1a	20			
	A2c	30	·0324		
	A3d	38			
	A2c	12	·0321		
	TOTAL	250	·1533	·1314	·0219
46	A1d	50	·0273		
	A1a	50	·0308		
	A2d	50	·0334		
	A2d	20			
	A3a	30	·0292		
	A3a	50	·0289		
	TOTAL	250	·1496	·1297	·0199
48	A1d	50	·0276		
	A1d	50	·0280		
	A2a	50	·0331		
	A2a	50	·0306		
	A3a	50	·0315		
	TOTAL	250	·1508	·1343	·0165
50	A3a	50	·0321		
	A3d	50	·0280		
	A2c	50	·0318		
	A1c	50	·0323		
	A1c	50	·0323		
	TOTAL	250	·1565	·1390	·0175
52	A3d	50	·0269		
	A3d	50	·0289		
	A2c	50	·0361		
	A1d	50	·0308		
	A1d	50	·0298		
	TOTAL	250	·1525	·1359	·0166
54	A3a	50	·0294		
	A3a	30			
	A2d	20	·0304		
	A2d	50	·0323		
	A1a	50	·0328		
	A3b	50	·0358		
	TOTAL	250	·1607	·1357	·0250
56	A3c	50	·0300		
	A3c	50	·0298		
	A3c	50	·0300		
	A2d	50	·0337		
	A3a	50	·0322		
	TOTAL	250	·1557	·1391	·0166
58	A1d	50	·0290		
	A2d	50	·0304		
	A3d	50	·0311		
	A3d	50	·0313		
	A1a	50	·0298		
	TOTAL	250	·1516	·1390	·0126
60	A3d	50	·0320		
	A2d	50	·0304		
	A1a	50	·0334		
	A1d	50	·0292		
	A1d	50	·0290		
	TOTAL	250	·1540	·1403	·0137

TABLE C

GROWTH OF TIMOTHY SEED

Weight in Milligrams at Two day Intervals after Pollination using seeds from Eleven plants of one Clone together with Percentage Composition of dry matter and water.

Days after Pollination on 29/1/51	Number of Seeds	FRESH WEIGHT Total	FRESH WEIGHT Average per seed	DRY WEIGHT Total	DRY WEIGHT Average per seed	Percentage of Fresh weight	WATER Total	WATER Average per seed	Percentage of Fresh weight
0	1000	28·7	·0287	9·2	·0092	32·1	19·5	·0195	67·9
2	500	18·3	·0366	6·8	·0136	37·2	11·5	·0230	62·8
4	500	22·3	·0446	8·3	·0166	37·2	14·0	·0280	62·8
6	250	31·2	·1248	10·9	·0436	34·9	20·3	·0812	65·1
8	"	61·1	·2444	18·7	·0748	30·6	42·4	·1696	69·4
10	"	65·6	·2624	21·8	·0872	33·2	43·8	·1752	66·8
12	"	98·4	·3936	33·6	·1344	34·2	64·8	·2592	65·8
14	"	119·9	·4796	48·1	·1924	40·1	71·8	·2872	59·9
16	"	132·5	·5300	62·0	·2480	46·8	70·5	·2820	53·2
18	"	153·6	·6144	78·7	·3148	51·2	74·9	·2996	48·8
20	"	164·0	·6560	92·4	·3696	56·3	71·6	·2864	43·7
22	"	174·4	·6976	101·0	·4040	57·9	73·4	·2936	42·1
24	"	187·7	·7508	112·3	·4492	59·8	75·4	·3016	40·2
26	"	197·3	·7892	123·1	·4924	62·4	74·2	·2968	37.6
28	"	200·4	·8016	131·7	·5268	65·7	68·7	·2748	34·3
30	"	202·5	·8100	131·5	·5260	64·9	71·0	·2840	35·1
32	"	202·8	·8112	135·0	·5400	66·6	67·8	·2712	33·4
34	"	203·2	·8128	137·7	·5508	67·8	65·5	·2620	32·2
36	"	199·6	·7984	141·5	·5660	70·9	58·1	·2324	29·1
38	"	189·7	·7588	138·8	·5552	73·2	50·9	·2036	26·8
40	"	175·1	·7004	134·8	·5392	77·0	40·3	·1612	23·0
42	"	156·3	·6252	131·9	·5276	84·4	24·4	·0976	15·6
44	"	153·3	·6132	131·4	·5256	85·7	21·9	·0876	14·3
46	"	149·6	·5984	129·7	·5188	86·7	19·9	·0796	13·3
48	"	150·8	·6032	134·3	·5372	89·1	16·5	·0660	10·9
50	"	156·5	·6260	139·0	·5560	88·8	17·5	·0700	11·2
52	"	152·5	·6100	135·9	·5436	89·1	16·6	·0664	10·9
54	"	160·7	·6428	135·7	·5428	84·4	25·0	·1000	15·6
56	"	155·7	·6228	139·1	·5564	89·3	16·6	·0664	10·7
58	"	151·6	·6064	139·0	·5560	91·7	12·6	·0504	8·3
60	"	154·0	·6160	140·3	·5612	91·1	13·7	·0548	8·9

TABLE D

Germination of Timothy Seed

Trial test of Fifty mature seeds to ascertain appropriate Testing time.

Number of days after placing in Incubator on 3rd April, 1951

Number of seeds germinating in specified number of days in Incubator

Plant	1	2	3	4	5	6	7	8
A1a	0	47	47	49	49	49	49	50

TABLE C

TABLE D

TABLE E

GERMINATION OF TIMOTHY SEED SAMPLES

Harvested at Different stages of Growth

Date of Pollination 29th January 1951. Date of Commencement of Germination tests, 9th April, 1951. Each sample tested comprised Fifty seeds.

Number of days from Pollination to Harvest	Number of Seeds Germinating after specified number of days in Incubator												Percentage Germination after 12 days
	1	2	3	4	5	6	7	8	9	10	11	12	
0 to 8	0	0	0	0	0	0	0	0	0	0	0	0	0
10	0	0	0	2	3	4	4	4	4	4	5	5	10
12	0	0	10	27	33	34	34	34	34	34	34	35	70
14	0	0	11	23	29	30	30	30	30	32	32	32	64
16	0	15	35	38	38	38	38	38	38	38	38	38	76
18	0	29	38	42	43	43	43	44	44	44	44	44	88
20	0	30	34	34	36	38	38	40	40	41	42	42	84
22	0	37	43	46	48	48	48	48	48	48	48	48	96
24	0	40	45	47	48	48	48	48	48	48	48	48	96
26	0	35	42	46	48	48	48	49	49	49	49	49	98
28	0	41	46	48	48	48	48	50	--	50	50	50	100
30	0	39	41	46	46	47	47	47	47	47	47	47	94
32	0	34	43	48	48	48	48	49	49	49	49	49	98
34	0	40	49	50	--	50	50	50	50	50	50	50	100
36	0	48	50	--	50	50	50	50	50	50	50	50	100
38	0	50	--	50	50	50	50	50	50	50	50	50	100
40	0	50	--	50	50	50	50	50	50	50	50	50	100
42	0	49	49	49	50	--	50	50	50	50	50	50	100
44	0	50	--	50	50	50	50	50	50	50	50	50	100
46	0	49	49	49	49	49	49	49	49	49	49	49	98
48 to 60	0	50	--	50	50	50	50	50	50	50	50	50	100

TABLE E.

TABLE F

GERMINATION OF TIMOTHY SEED

Observation on the Condition of Seeds not Germinating in the Normal Testing Period.

Number of days from Pollination to Harvest	Seeds Germinating within 12 days	Number of days in Incubator	Number of Seeds Germinating	Total seeds Germinating before being chilled	Condition of seeds ungerminated after 43 days in Incubator: Soft Yellow seeds	Soft Black seeds	Total soft ungerminated seeds	Firm seeds ungerminated before Chilling	Results of Chilling Trials upon Firm Ungerminated Seeds: seeds germinating at 1st chilling	seeds germinating at 2nd chilling	seeds germinating at 5th chilling	Firm seeds ungerminated after 7th Period of Chilling	Grand total of seeds germinating within 12 days, as feeble Late seeds & as chilled seeds
0 to 8	0*				50		50						0
10	5			5	45		45						5
12	35	42	1	36	4	1	5	9	3	2		4	41
14	32	42	1	33	6	3	9	8	4	2	1	1	40
16	38	43	1	39	4	4	8	3			1	2	40
18	44	42	1	45	1	2	3	2	2				47
20	42	43	2	44	1	3	4	2				2	44
22	48	19	1	49		1	1						49
24	48			48	1		1	1	1				49
26	49	41	1	50									50
28	50			50									50
30	47			47	1		1	2	2				49
32	49			49				1			1		50
34 to 44	50			50									50
46	49			49		1	1						49
48 to 60	50			50									50

* Fifty seeds removed from the heads collected at two day intervals during the Weight Growth Test were tested for their germinating capacity. Thus in order to obtain the percentages of the above numbers it is necessary to multiply by two.

TABLE F

TABLE G

SEEDLING GROWTH FROM CHILLED TIMOTHY SEED

Seeds chilled at 3°c. for Seven Days except for the Fifth Chilling, which was carried on for over Two months. (See Table X on Page) The Chilled Seeds were subsequently incubated at 20°c.

Maximum and mean lengths in Millimeters of the Vegetative shoots of Seedlings Developing from Samples Designated by Period in Days from Pollination to Harvesting

	12	14	16	18	20	24	30	32
After First Chilling	20	35		44		49	53	
" " "	18	32		43			52	
" " "	17	31						
" " "		26						
Average of First Chilling	18°3	31		43°5		49	52°5	
After Second Chilling	21	27						
" " "	(4*)	23						
Average of Second Chilling	21*	25						
After Third Chilling	No seeds Germinated							
After Fourth Chilling	No seeds Germinated							
After Fifth Chilling		35	35					53
After Sixth Chilling	No seeds Germinated							
After Seventh Chilling	No seeds Germinated							
Grand Average of all Chillings	19	29°9	35	43°5		49	52°5	53

TABLE G.

TABLE H

WEATHER REPORT

Observations recorded at the Seed Testing Station during the Flowering of Timothy

Date	
January 1951	
19	Overcast, mild. Windy in afternoon. Flowering commenced on some tagged heads of plant A 1 b.
20	Sunny, hot at 9 a.m. No flowering, although stigmas showing on "Green" head of plant A 1 b.
21	Hot, sunny, windy. Some heads of plant A 1 b must have flowered late on January 20.
22	Overcast, windy. Intermittent sun.
23	Rain showers, heavy cloud. Mild
24	Heavy overcast with thunderstorm in afternoon. Humid.
25	Heavy overcast, steady rain. Humid.
26	Overcast, occasional light showers. Cooler.
27	Overcast in morning, cool. Sunny and warmer in afternoon.
28	Partial cloud, cool.
29	Clear, sunny.
30	Overcast, cool.
31	Overcast, cool.
February 1951	
1	Sunny, cool, windy.
2	Sunny, cool, windy.
3	Sunny, warm.
4	Sunny, warm
5	Hot, sunny.
6	Cloudy, warm.
7	Overcast.
8	Sunny, warm.
	Flowering ended on tagged heads of plant B1b.

T A B L E I

RECORD OF TEMPERATURES

Daily Minimum and Maximum Shade Temperatures in the Open and in the Greenhouses at Grasslands Division during the Investigations on Flowering and Seed Growth

All Temperatures were taken at 9 a.m. with a Maximum-minimum Thermometer.

Flowering of Timothy Experiment was undertaken at the Seed Testing Station from January 19th until February 8th.

Growth of Timothy Experiment was carried on at Grasslands Division from Pollination on January 29th until March 30th .

D A T E		J A N U A R Y													F E B R U A R Y															
		19	20	21	22	23	24	25	26	27	28	29	30	31	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Shade Temperatures in the Open	Minimum	56	54	53	62	62	64	57	51	47	60	46	56	58	52	54	57	53	52	60	54	46	58	55	52	57	54	54	57	56	58	59	52
	Maximum	69	74	75	77	71	79	62	62	63	66	69	71	68	65	68	69	77	79	72	67	76	69	72	70	71	69	76	70	73	76	72	75

D A T E		F E B R U A R Y									M A R C H															
		20	21	22	23	24	25	26	27	28	1	2	3.	4	5	-6	7	8	9	10	11	12	13	14	15	16
Shade Temperatures in the Open	Minimum	58	43	53	46	46	55	57	59	61	65	62	57	60	61	60	55	58	53	57	56	54	51	56	51	60
	Maximum	65	76	74	80	75	77	76	75	74	82	71	76	78	73	75	80	76	78	77	68	69	70	67	62	70
Temperatures in Red Clover Green-House	Minimum	61	54	57	54	54	61	61	61	63	67	64	62	64	65	64	60	63	60	61	62	56	58	56	53	54
	Maximum	82	71	79	75	77	78	78	75	75	76	76	72	74	76	74	74	78	74	76	74	68	72	69	66	72

D A T E		M A R C H															A P R I L									
		17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	1	2	3	4	5	6	7	8	9	10
Shade Temperatures in the Open	Minimum	42	46	48	56	50	44	56	55	51	52	52	55	47	49	44	57	56	59	59	53	53	53	58	56	54
	Maximum	72	73	75	70	70	71	69	65	69	64	62	69	70	71	53	70	73	68	68	69	68	75	72	70	62
Temperatures in Timothy Greenhouse	Minimum	46	49	51	58	51	48	58	57	52	53	52	54	50	52	50	58	59	60	60	56	54	56	60	58	56
	Maximum	88	88	88	88	76	83	79	78	82	80	84	78	86	84	82	90	76	87	74	80	88	92	95	93	85

TABLE J

RECORD OF RELATIVE HUMIDITY

Conditions in the Timothy Greenhouse at Grasslands Division

The relative humidity fell rapidly each day from a maximum of 83% to 90% during the night and early morning to a minimum of 42% to 60% at approximately 3 P.M. From 3 P.M. until 10 P.M. there was a rapid increase in the relative humidity, although this increase was not as rapid as was the decrease in the morning. From 10 P.M. until 8 A.M. or 10 A.M. the humidity remained approximately the same. The maximum varied only 7 degrees between March 22 and 29, but the minimum varied by 18 degrees.

Percentage Relative Humidity*
March 1951

Date	22	23	24	25	26	27	28	29
Time	3 P.M.	Noon	2:30	3 P.M.	3 P.M.	4 P.M.	3 P.M.	12:30
Minimum	52	60	56	46	45	53	42	44
Time	2 A.M.	10 P.M.	7A.M.	3 A.M.	5 A.M.	8 A.M.	8 A.M.	
Maximum	88	88	85	86	90	83	90	

* A record of the Relative Humidity was recorded for only one week because the hygrograph was mainly used by the writer in another greenhouse on an experiment with Montgomeryshire Red Clover that was being carried out concurrently with the Timothy experiment.